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Original Article

Significance of TRIM29 and β-catenin expression in non-small-cell lung cancer

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Abstract

Background: TRIM29 belongs to the tripartite motif (TRIM) protein family. It has been reported to be up-regulated or be down-regulated in many cancer types, suggesting the oncogenic function of TRIM29 may be depend on different molecular signaling pathway. It was found that β-catenin function (a key molecule in the Wnt signaling pathway) was required for TRIM29's oncogenic effects. TRIM29 gene expression was also found to be heterogeneous in non-small-cell lung cancer (NSCLC) subtypes. In this study, the possible associations of TRIM29 expression with clinicopathological factors, prognosis, and β-catenin in human NSCLC were analyzed.

Methods: TRIM29 and β-catenin expression of tumor and adjacent normal tissues in 251 cases of NSCLC treated by surgery was detected by the Immunohistochemical method. The relationship between clinical pathological data, β-catenin, and TRIM29 expression was analyzed.

Results: TRIM29 expression of tumor tissues was significantly higher than adjacent normal tissues. Expression of TRIM29 in squamous cell carcinoma (SC) tissues was positively correlated with abnormal expression of β-catenin, histological grade, tumor-node-metastasis (TNM) stage, and lymph node metastasis and that was positively correlated with tumor size, histological grading, TNM stage and lymph node metastasis in adenocarcinoma (AC). TRIM29 expression in SC and AC was significantly different and the intensity of poorly differentiated SC was significantly higher than that of AC. High-expression of TRIM29, poorly differentiated grade, and high clinical stage were independent prognostic indicators.

Conclusion: We considered that TRIM29 may play a reference role in distinguish poorly differentiated AC and SC of NSCLC, combining with CK5/6 and CK7, and it could improve postoperative assessment and have the reference value for clinical treatment. The interaction between TRIM29 and β-catenin may participate in the development of lung SC.

Keywords: β-catenin; pathologic diagnosis; prognosis; NSCLC; TRIM29

1. Introduction

Lung cancer is the leading cause of cancer-related death in the United States, only 15% of all lung cancer patients live 5 years or more after diagnosis.[1] Rapidly metastasis and spread are important reasons for the low 5-year survival rate of lung cancer. The prediction of clinical prognosis still depends on conventional pathologic variables such as tumor size, tumor grade, lymph node, and clinical stage. It is of great clinical value to find sensitive and specific early biomarkers for the diagnosis and prognosis of this malignancy, as well as novel therapeutic strategies. Non-small-cell lung cancer (NSCLC), the predominant form of lung cancer, formerly viewed as a frequent unique disease of uniform presentation, is now viewed as a mosaic of diseases defined by pathological characteristics or molecular characteristics. It consists of two major histological subtypes: squamous cell carcinoma (SC) and adenocarcinoma (AC). The subclasses differ in their clinical biological behavior, with AC tending to worse outcomes than SC.[2] Currently, histological type has been an important factor in selecting treatment among NSCLC, because novel molecularly targeted therapies have shown different activities in histological subclasses.[3] Therefore, the

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histological subtype must be correctly identified in the pathological diagnosis of NSCLC. Recently, TRIM29 gene expression was found to be heterogeneous in NSCLC subtypes according to gene-expression profiles.[4] Initially known as the ataxia telangiectasia group D complementing gene (ATDC), TRIM29 was cloned from ataxia telangiectasia group D fibroblasts as a gene with the ability to complement sensitivity to ionizing radiation. [5]

TRIM29, located at chromosome 11q23, is expressed normally in placenta, lung, thymus, prostate, testis, and colon, while it was undetectable in heart, brain, skeletal muscle, pancreas, spleen, ovary, or small intestine.[6] It belongs to the tripartite motif (TRIM) protein family. This family consists of at least 37 member proteins and is characterized by its unique structure: three zinc-binding domains (RING, B-box type 1, and B-box type 2), followed by a coiled-coil region. While some of the domains may be absent or present (TRIM29 contains the B1-B2-CC domains but lacks the R domain),[7] TRIM family has been implicated in a variety of cellular processes, such as development and growth. TRIM29 has been reported to be up-regulated in a number of cancer types and to also be down-regulated in some other cancer types suggesting the function of TRIM29 may be depend on different cellular context and molecular signaling pathway.[8] Wang[8] found that TRIM29 expression correlated with elevated β-catenin levels in pancreatic cancer, and β-catenin function (a key molecule in Wnt signaling pathway) was required for TRIM29's oncogenic effects. However, both TRIM29 and β-catenin gene expression in NSCLC have not been reported.

In this study, we explored TRIM29 and β-catenin gene expression in human NSCLC. The possible associations of TRIM29 gene expression with clinicopathological factors, prognosis, and β-catenin were also analyzed.

2. Methods

2.1. Patients

Retrospective analysis was performed on NSCLC patients who had undergone surgery between January 2007 and December 2009 in our department of Wuxi People's Hospital Affiliated to Nanjing Medical University. The median age of the patients was 62 years, ranging from 33–84 years. There were 188 males and 63 females (2.98:1). According to World Health Organization (WHO) standards, there were 127 cases of SC and 124 cases of AC, well-differentiated = 24, differentiated = 118, and poorly differentiated = 109. According to tumor-node-metastasis (TNM) stage (the 7th edition of TNM staging of the Union for International Cancer Control and American Joint Committee on Cancer in 2009), there were 140 (55.8%) in staging level Ⅰ-Ⅱ and 111 (44.2%) in staging level Ⅲ-Ⅳ. A total of 131 (52.2%) patients were positive for lymph node metastasis, while 120 (47.8%) were negative. No adjuvant radiotherapy or chemotherapy was administered before surgery. One representative block was selected for immunohistochemical study. The study was permitted by the patients or the family members of patients.

2.2. Immunohistochemistry

Paraffin slices were treated according to the EnVision immunohistochemical kit (Maxim Biotechnology Ltd, Fuzhou, China), and results were analyzed using a double-blind method. Five high-power fields (×400) were selected at random, and two pathologists evaluated scores independently. Phosphate buffered saline (PBS), instead of the primary antibody, was used as negative control. cytokeratin (CK)7 (OV-TL 12/30 + 72.2, Labvision, Labvision Co, Kalamazoo, USA) and CK5/6(D5/16B4, Labvision, USA) expression were detected, CK5/6 marked for SC, and CK7 marked for AC. TRIM29 (polyclonal antibodies, Lifespan Inc, Seattle, WA, USA) expression was scored according to the intensity of the dye color and the number of positive cells. The intensity of the dye color was graded as 0 (no color), 1 (light yellow), 2 (light brown), or 3 (brown), and the number of positive cells was graded as 0 (<5%), 1 (5%–25%), 2 (25%–50%), 3 (51%–75%), or 4 (>75%). The two grades were added together and specimens were assigned to one of 4 levels: 0-1 score (−, negative), 2 scores (+, weakly positive), 3-4 scores (++, positive), and more than 5 scores (+++, strongly positive).

2.3. Evaluation of staining of β-catenin (CAT-5H10, Labvision)

For counting cells with nuclear and/or cytoplasm staining, three microscopic fields with 200-fold magnification were randomly chosen. Evaluation of staining was carried out based on whether nuclear and/or cytoplasmic staining is detectable. Under microscopic field of 200-fold magnification, tissues are positive for β-catenin abnormal expression, if more than 10% of cells show cytoplasmic and/or nuclear staining.

2.4. Statistical analysis

All data were analyzed using SPSS 11.5 (SPSS Inc, IL, USA). χ^2 tests (or Fisher's exact test) were used for samples classified as percentages; Spearman analysis was used to determine the correlation between two variables. Both the Kaplan-Meier method and log-rank test were used for single variant analysis, and a Cox model was used to analyze relationships between survival rates and multiple variables. A p value < 0.05 were considered to be significant.

3. Results

3.1. Association between TRIM29 and β-catenin expression and clinicopathological factors in lung SC

Among 127 cases of lung SC, weakly positive, positive and strongly positive were 11 (8.7%), 55 (43.3%) and 61(48.0%) cases respectively (Fig. 1), and there was no negative case. Only 17 cases (13.4%) were weakly positive in adjacent normal lung tissue (≥10 cm distant from cancer), the rest belonging to negative. TRIM29 expression of tumor tissue was significantly higher ($p < 0.05$). Furthermore, TRIM29 expression was

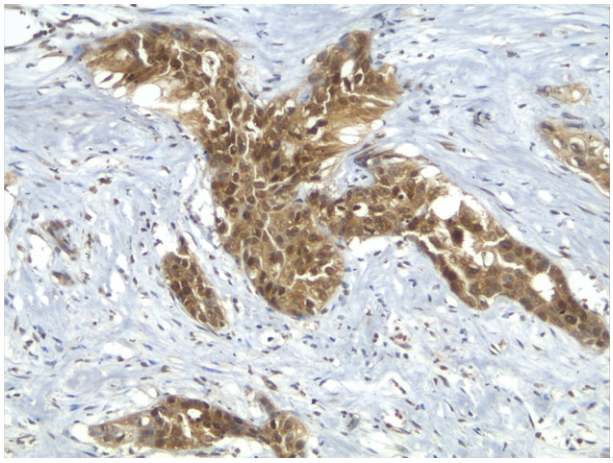

Fig. 1. Strong staining of TRIM29 in lung poorly differentiated SC. SC = squamous cell carcinoma.

positively correlated with abnormal expression of β-catenin, histologic grade (Figs. 2 and 3), TNM stage and lymph node metastasis, while not correlated with sex, age, and tumor size (Table 1).

3.2. Association between TRIM29 and β-catenin expression and clinicopathological factors in lung AC

Among 124 cases of lung AC, negative, weakly positive and positive were 25 (22.2%), 84 (67.7%) and 15 (12.1%) cases respectively (Fig. 4), and there was no strong positive case. In the adjacent normal lung tissue only 11(8.9%) cases were weak positive, the rest being negative. TRIM29 expression in lung AC tumor tissue was also significantly increased ($p < 0.05$). Simultaneously, TRIM29 expression was positively correlated with tumor size, histologic grade, TNM stage, and lymph node metastasis, and not correlated with sex, age and abnormal expression of β-catenin (Table 2).

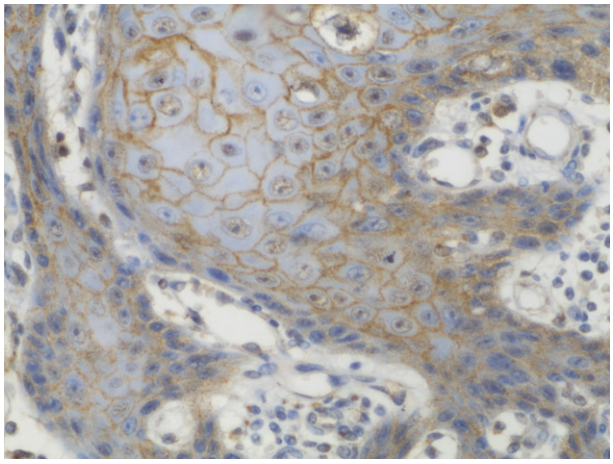

Fig. 2. β-catenin membrane staining in well-differentiated lung SC. SC = squamous cell carcinoma.

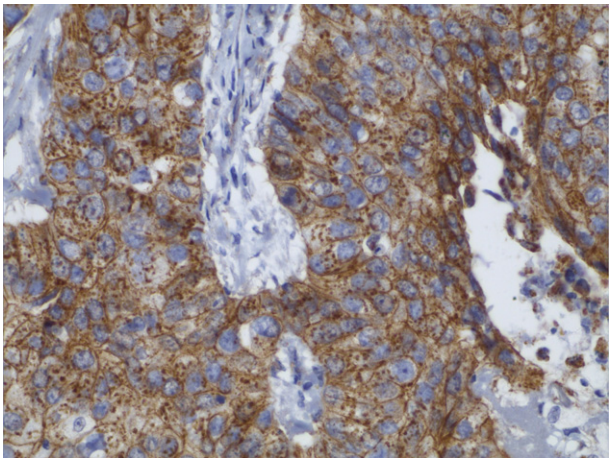

Fig. 3. β-catenin cytoplasm and nuclear staining poorly differentiated SC. SC = squamous cell carcinoma.

3.3. Difference of TRIM29 expression in lung SC and AC

In Table 3, we found that TRIM29 expression in lung SC and AC was significantly different. Tables 1 and 2 showed that TRIM29 expression of SC was significantly higher in poorly differentiated tumor, with strongly positive (62.0%) or positive (35.2%), while AC was significantly weaker, with the majority being weakly positive (74.4%) and no strong positive.

3.4. Association between TRIM29 expression and prognosis in lung SC patients

In view of the closely correlation between TRIM29 and lung SC, we conducted TRIM29 survival analysis on 56 cases of lung SC with follow-up data. The 1-year and 3-year survival rates of 56 cases were 84.2% and 53.4%, respectively. The survival rate was significantly lower in the higher TRIM29 expression group ($p < 0.01$). These results are shown in Fig. 5. By log-rank test, higher expression of TRIM29, higher histologic grade, and TNM stage, and lymph node metastasis showed

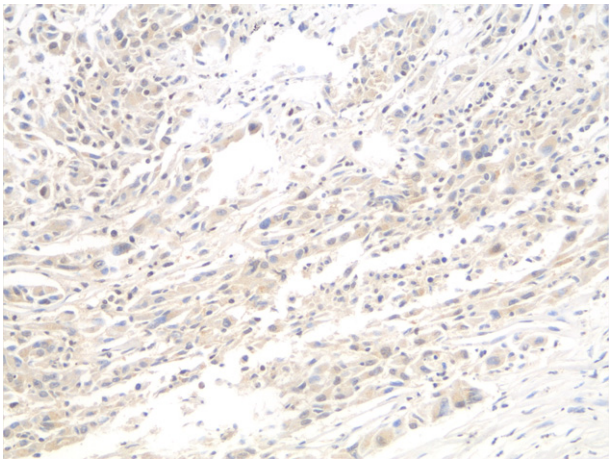

Fig. 4. Light staining of TRIM29 in lung poorly differentiated AC. AC = adenocarcinoma.

Table 1
Correlation of TRIM29 expression and clinicopathological factors and β-catenin expression in lung SC.

		TRIM29 expression intensity				*p* value
		-	+	++	+++	
β-catenin abnormal expression	Negative	0	7	17	14	0.030
	Positive	0	4	38	47	
Age, y	<60	0	0	4	5	0.499
	≥60	0	11	51	56	
Sex	Male	0	7	47	58	0.772
	Female	0	4	8	3	
Tumor size	≤5 cm	0	8	36	36	0.330
	>5 cm	0	3	19	25	
Clinical stage	I–II	0	10	42	24	<0.001
	III–IV	0	1	13	37	
Pathological grade	I	0	3	2	0	<0.001
	II	0	6	28	17	
	III	0	2	25	44	
Lymph node metastasis	Positive	0	10	41	13	<0.001
	Negative	0	1	14	47	

SC = squamous cell carcinoma.

worse prognosis ($p < 0.05$), and age, sex, and tumor size had no prognostic significance. Univariate analysis showed that the following factors were significantly related to postoperative survival: high TRIM29 expression, histologic grade and TNM clinical stage ($p < 0.05$). These results are shown in Table 4.

4. Discussion

TRIM29 encodes a 588 amino acid protein with multiple zinc-finger motifs and an adjacent leucine-zipper motif and thus may act as a transcriptional regulatory factor.[9] TRIM29 has been reported to be overexpressed in bladder,[10] colorectal,[11] ovarian,[12] and endometrial cancers[13] and in multiple myeloma,[14] with apparent reduced expression in breast,[15] and prostate cancers,[16] suggesting that the function of TRIM29 may be dependent on different cellular context and molecular signaling pathway. Using immunohistochemical analysis, we found TRIM29 protein was up-regulated in NSCLC than adjacent normal tissues and correlated with clinicopathological factors and prognosis, therefore speculating TRIM29 gene over-expression may be involved in the pathogenesis of NSCLC. Also, we found that TRIM29 over-expression and abnormal expression of β-catenin was significantly correlated in SC, but not in AC. Currently, many literatures had reported that the abnormal expression of β-catenin play an important role in the pathogenesis of NSCLC,[17] but the correlation between TRIM29 and β-catenin gene has not yet been reported. Wang[8] found that TRIM29 as a protein highly expressed in the majority of human pancreatic adenocarcinomas. Additionally, elevated levels of TRIM29 expression correlated with elevated β-catenin levels in pancreatic cancer cell lines and primary pancreatic cancers, and silencing of TRIM29 via shRNA approaches antagonized β-catenin/T cell factor (TCF)-mediated reporter activation and activation of TCF target genes. Gene β-catenin was implicated in the oncogenic effects of TRIM29 *in vitro* and *in vivo*, and the ability of TRIM29 to increase β-catenin levels appeared to be attributable to TRIM29's effects on disheveled-2 protein expression. TRIM29 is an important positive regulator of β-catenin-dependent signaling in pancreatic cancer. Our results indicated that lung SC (not lung AC) and pancreatic AC might exist similar molecular signal pathogenesis involving relationship between TRIM29 and β-catenin. The molecular mechanisms need further study.

By real-time reverse transcriptase-polymerase chain reaction, Kosaka and colleagues[7] analyzed TRIM29 mRNA expression status with respect to various clinicopathological parameters in 124 patients with gastric cancer. Results indicated that the expression of TRIM29 was far higher in gastric cancer tumor tissue than in surrounding tissue. Increased

Table 2
Correlation of TRIM29 expression and clinicopathological factors and β-catenin expression in lung AC.

		TRIM29 expression intensity				*p* value
		-	+	++	+++	
β-catenin abnormal expression	Negative	9	22	1	0	0.051
	Positive	16	62	14	0	
Age, y	<60	1	7	2	0	0.295
	≥60	24	77	13	0	
Sex	Male	16	45	15	0	0.594
	Female	9	39	0	0	
Tumor size	≤5 cm	24	68	10	0	0.016
	>5 cm	1	16	5	0	
Clinical stage	I–II	22	40	2	0	<0.001
	III–IV	3	44	13	0	
Pathological grade	I	10	9	0	0	<0.001
	II	13	46	7	0	
	III	2	29	8	0	
Lymph node metastasis	Positive	3	49	15	0	<0.001
	Negative	22	35	0	0	

AC = adenocarcinoma.

Table 3
Difference of TRIM29 expression in lung SC and AC.

	TRIM29 expression intensity				*p* value
	-	+	++	+++	
Lung AC	25	84	15	0	<0.001
Lung SC	0	11	55	61	

AC = adenocarcinoma; SC = squamous cell carcinoma.

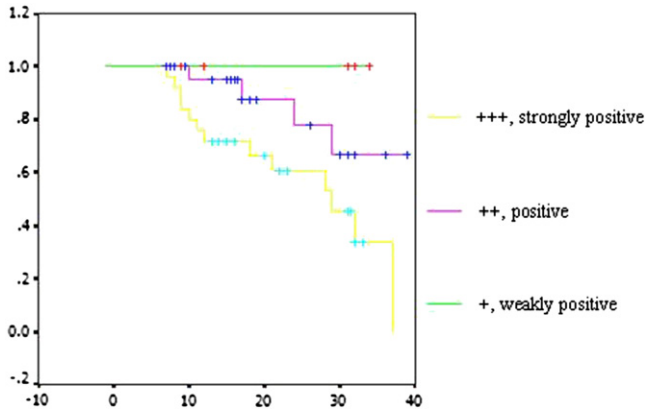

Fig. 5. TRIM29 survival analysis on 56 cases of lung SC. SC = squamous cell carcinoma.

Table 4
Multiple factor cox model regression variable form of lung SC.

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B)	
							Lower	Upper
TRIM29 Expression Intensity	1.668	0.697	5.727	1	0.017	5.300	1.352	20.774
Clinical stage	2.079	0.667	9.707	1	0.002	7.993	2.162	29.553
Pathologic grade	1.325	0.583	5.159	1	0.023	3.760	1.199	11.793

B = partial regression coefficient; CI = confidence interval; df = degree of freedome; Exp(B) = hazard ratio; SC = squamous cell carcinoma; SE = standard error; Sig. = significance.

TRIM29 messenger RNA (mRNA) expression was markedly associated with such parameters as histological grade, large tumor size, and extent of tumor invasion, and it was an independent predictor for lymph node metastasis. In the TRIM29 high-expression group, furthermore, patients with high TRIM29 mRNA expression showed a far poorer survival rate. In pancreatic cancer cells, expression of TRIM29 promoted cellular proliferation and enhanced tumor growth and metastasis.[8] To investigate the biological role of TRIM29 expression in NSCLC, using immunohistochemistry (IHC), we found that TRIM29 expression was positively correlated with histologic grade, TNM stage, and lymph node metastasis in lung SC and was positively correlated with tumor size, histologic grade, TNM stage, and lymph node metastasis in lung AC. Univariate analysis showed that the following factors were significantly related to postoperative survival in lung SC: TRIM29 high expression, histologic grade and TNM clinical stage. Our results strongly suggest TRIM29 could be a valuable biomarker for the prediction of NSCLC prognosis. Lymph node metastasis is usually a reliable prognostic indicator, but it did not enter the multi-factor Cox model in this study, although the log-rank test suggested its positive significance. We analysis that skip metastases and micrometastases, easily leading to missed diagnosis in routine pathology work, might affect the statistical results in addition to the limited number of follow-up cases. Tumor stage is the prognostic factor; however, it is relatively hysteretic for prognosis. So, high expression of TRIM29 (particularly strong positive of lung SC and positive of AC) could lead to clinical attention in spite of no lymph node metastasis. Currently, further studies are needed on molecular mechanism of TRIM29 promoting invasion and metastasis and investigations of whether TRIM29 could be used as a target for novel therapeutic approaches in NSCLC.

NSCLC includes two major histological subtypes: SC and AC. Traditionally, histological classification of NSCLC is carried out by the standard histochemical staining using hematoxylin and eosin. However, it is difficult to see the typical histological structures in poorly differentiated tumor. Moreover, the increased use of limited bronchoscopic or needle biopsies available for diagnosis increase the difficulty of histological type and there is a relatively high rate of morphological misdiagnosis in needle biopsy samples compared to surgical specimens.[18] Immunohistochemistry could improve pathological diagnosis with a combination of AC markers (CK7) and SC markers (p63 and CK5/6). However, the specificity of each marker for one particular histological subtype remains low.[19] We found that there was significant difference of TRIM29 expression between lung SC and AC. Particularly in poorly differentiated tumor, TRIM29 expression of SC was significantly higher, with strongly positive (62.0%) and positive (35.2%), while AC was significantly weaker, with the majority being weakly positive (74.4%) and no strong positive. Therefore, we considered that TRIM29 may play a reference role in distinguish poorly differentiated AC and SC of NSCLC, combining with CK5/6 and CK7.

In conclusion, by immunohistochemistry, this study indicated that TRIM29 and β-catenin (a key molecule of Wnt signaling pathway) interactions may play an important role in the pathogenesis of lung SC, and TRIM29 biomarker has the application value in the pathological diagnosis and clinical pathology of NSCLC. We recommend that routine pathological diagnosis of lung cancer could combine the immune markers TRIM29, improving the accuracy of pathological diagnosis and having guiding values for clinical staging, treatment and survival assessment.

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